

We claim:

1. A protein-free defined medium for culturing epidermal keratinocytes comprising:

- a) N- (2-OH-ethyl-)piperazine-N -(2-ethane-sulfonic acid) at a concentration of 14-22 mM;
- b) sodium chloride at a concentration of 100-120 mM;
- c) histidine at a concentration of 0.1-0.25 mM;
- d) isoleucine at a concentration of 0.05-0.5 mM;
- e) methionine at a concentration of 0.1-0.5 mM;
- f) phenylalanine at a concentration of 0.1-0.5 mM;
- g) tryptophan at a concentration of 0.05-0.5 mM;
- h) tyrosine at a concentration of 0.1-0.5 mM; and
- i) retinyl acetate at a concentration of 0.3-33 ng/ml.

2. A protein-free defined medium for culturing epidermal keratinocytes comprising:

- a) N- (2-OH-ethyl-)piperazine-N'-(2-ethane-sulfonic acid) at a concentration of 14-22 mM;
- b) sodium chloride at a concentration of 100-120 mM;
- c) calcium²⁺ ions at a concentration of 0.7-3.0 mM;
- d) histidine at a concentration of 0.1-0.25 mM;
- e) isoleucine at a concentration of 0.05-0.5 mM;
- f) methionine at a concentration of 0.1-0.5 mM;
- g) phenylalanine at a concentration of 0.1-0.5 mM;
- h) tryptophan at a concentration of 0.05-0.5 mM;
- i) tyrosine at a concentration of 0.1-0.5 mM;
- j) β -transforming growth factor at a concentration of 3.0-30 ng/ml; and

k) retinyl acetate at a concentration of 0.3-33 ng/ml.

3. A protein-free defined medium for culturing epidermal keratinocytes comprising:

- a) N-(2-OH-ethyl)-piperazine-N'-(2-ethane-sulfonic acid) at a concentration of 14-22 mM;
- b) sodium chloride at a concentration of 100-120 mM;
- c) calcium.sup.2+ ions at a concentration of 0.7-3.0 mM;
- d) histidine at a concentration of 0.1-0.25 mM;
- e) isoleucine at a concentration of 0.05-0.5 mM;
- f) methionine at a concentration of 0.1-0.5 mM;
- g) phenylalanine at a concentration of 0.1-0.5 mM;
- h) tryptophan at a concentration of 0.05-0.5 mM;
- i) tyrosine at a concentration of 0.1-0.5 mM;
- j) linoleic acid at a concentration of 1-15 ng/ml; and
- k) retinyl acetate at a concentration of 0.3-33ng/ml.

4. A method for the formation of a histologically complete stratified epidermis comprising the steps of:

a) isolation of basal stem cells from animal epithelium using a solution comprising:

- i) glucose at a concentration of about 10 mM;
- ii) N-(2-OH-ethyl)-piperazine-N'-(2-ethanesulfonic acid) at a concentration of 16-22 mM;
- iii) sodium chloride at a concentration of 90-140 mM;
- iv) potassium chloride at a concentration of about 3 mM;
- v) sodium orthophosphate (Na.sub.2 HPO.sub.4.7H.sub.2 O) at a concentration of 1 mM;
- vi) phenol red at a concentration of 0.0033 mM;

- vii) about 100 units of penicillin per ml of solution;
- viii) about 100 units of streptomycin per ml of solution; and
- ix) trypsin at a concentration of 0.1%-0.2% w/v;

b) recovering said isolated basal stem cells using a solution comprising:

- i) glucose at a concentration of about 10 mM;
- ii) N-(2-OH-ethyl)-piperazine-N'-(2-ethanesulfonic acid) at a concentration of 16- 22 mM;
- iii) sodium chloride at a concentration of 90-140 mM;
- iv) potassium chloride at a concentration of about 3 mM;
- v) sodium orthophosphate (Na.sub.2 HPO.sub.4.7H.sub.2 O) at a concentration of 1 mM;
- vi) phenol red at a concentration of 0.0033 mM;
- vii) about 100 units of penicillin per ml of solution;
- viii) about 100 units of streptomycin per ml of solution; and
- ix) soy bean trypsin inhibitor at a concentration of 0.1%-1.0% w/v;

c) culturing said isolated basal stem cells in a medium to form a confluent sheet of undifferentiated epithelial tissue, said medium comprising:

- i) N-(2-OH-ethyl)-piperazine-N'-(2-ethanesulfonic acid) at a concentration of 14-22 mM;
- ii) sodium chloride at a concentration of 100-120 mM;
- iii) histidine at a concentration of 0.1-0.25 mM;
- iv) isoleucine at a concentration of 0.05-0.5 mM;
- v) methionine at a concentration of 0.1-0.5 mM;
- vi) phenylalanine at a concentration of 0.1-0.5 mM;

vii) tryptophan at a concentration of 0.05-0.5 mM;

viii) tyrosine at a concentration of 0.1-0.5 mM; and

ix) retinyl acetate at a concentration of 0.3-33 ng/ml;

d) culturing said sheet of undifferentiated epithelial tissue in a differentiation medium to form a sheet of differentiated and stratified tissue, said differentiation medium comprising:

i) N-(2-OH-ethyl-)piperazine-N'-(2-ethanesulfonic acid) at a concentration of 14-22 mM;

ii) sodium chloride at a concentration of 100-120 mM;

iii) calcium²⁺ ions at a concentration of 0.7-3.0 mM;

iv) histidine at a concentration of 0.1-0.25 mM;

v) isoleucine at a concentration of 0.1-0.5 mM;

vi) methionine at a concentration of 0.1-0.5 mM;

vii) phenylalanine at a concentration of 0.1-0.5 mM;

viii) tryptophan at a concentration of 0.05-0.5 mM;

ix) tyrosine at a concentration of 0.1-0.5 mM;

x) β -transforming growth factor at a concentration of 3.0-30 ng/ml; and

xi) retinyl acetate at a concentration of 0.3-33 ng/ml; and

e) culturing said differentiated and stratified tissue in a cornification medium to form a cornified epithelium, said cornification medium comprising:

i) N-(2-OH-ethyl-)piperazine-N'-(2-ethanesulfonic acid) at a concentration of 14- 22 mM;

ii) sodium chloride at a concentration of 100-120 mM;

iii) calcium²⁺ ions at a concentration of 0.7-3.0 mM;

iv) histidine at a concentration of 0.1-0.25 mM;

- v) isoleucine at a concentration of 0.05-0.5 mM;
- vi) methionine at a concentration of 0.1-0.5 mM;
- vii) phenylalanine at a concentration of 0.1-0.5 mM;
- viii) tryptophan at a concentration of 0.05-0.5 mM;
- ix) tyrosine at a concentration of 0.1-0.5 mM;
- x) linoleic acid at a concentration of 1-15 ng/ml;
- xi) retinyl acetate at a concentration of 0.3-33 ng/ml.

5. The method according to claim 4 wherein said histologically complete epidermis is human epidermis.

6. The method according to claim 4, further comprising the step of using serum or tissue extract or animal derived factors or other xenobiotics in combination with Ca^{2+} ions (1-2 mM) in order to form a stratified keratinizing epithelium.